

GENE REGULATION AND *IN VIVO* FUNCTION OF LIVER TRANSCRIPTION FACTORS

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MECHANISM OF HEPATOCYTE-SPECIFIC GENE TRANSCRIPTION

Hepatocyte Transcription Factor Families

The liver performs essential functions in the body by uniquely expressing hepatocyte-specific genes encoding plasma proteins, and enzymes involved in gluconeogenesis, glycogen storage, glucose metabolism, cholesterol homeostasis, and synthesis of bile salts (1). Functional analysis of numerous hepatocyte-specific promoter and enhancer regions reveals that they are composed of multiple *cis*-acting DNA sequences that bind different families of hepatocyte nuclear factors (HNFs) (2). Although none of these transcriptional regulatory factors is entirely liver-specific, the requirement for combinatorial protein interactions to achieve high transcriptional levels plays an important role in maintaining hepatocyte-specific gene expression (2,3). Isolation of the complementary DNA (cDNA) clones encoding these transcription factors facilitated the identification of their DNA binding and transcriptional activation domains. On the basis of homology within DNA binding domains, the transcription factors were grouped into related protein families, which include the winged helix HNF-3 α ,

-3 β , and -3 γ proteins (4,5); the cut-homeodomain HNF-6 (OC-1) and one cut-2 (OC-2) proteins (6–10), the orphan steroid hormone receptors HNF-4 α (11), and fetoprotein transcription factor (FTF) proteins (12,13), the POU-homeodomain HNF-1 α and vHNF-1 proteins (14–16), and the nkx homeodomain nkx-2.8 protein (17), the basic region leucine zipper (bZIP) CCAAT/enhancer binding proteins (C/EBPs) (18,19), and the related bZip family member albumin D-site-binding protein (DBP), which contains an additional proline and amino acid-rich (PAR) region in the DNA-binding domain (20–22).

Transthyretin DNA Control Region as a Model for Hepatocyte-Specific Gene Regulation

We have utilized the DNA regulatory regions of the transthyretin (TTR) gene, which encodes the serum carrier protein of thyroxine and vitamin A (23), as a model to understand hepatocyte-specific gene transcription. Studies of TTR suggest that hepatocyte-specific gene transcription is dependent on combinatorial interactions of multiple DNA binding sites by several distinct families

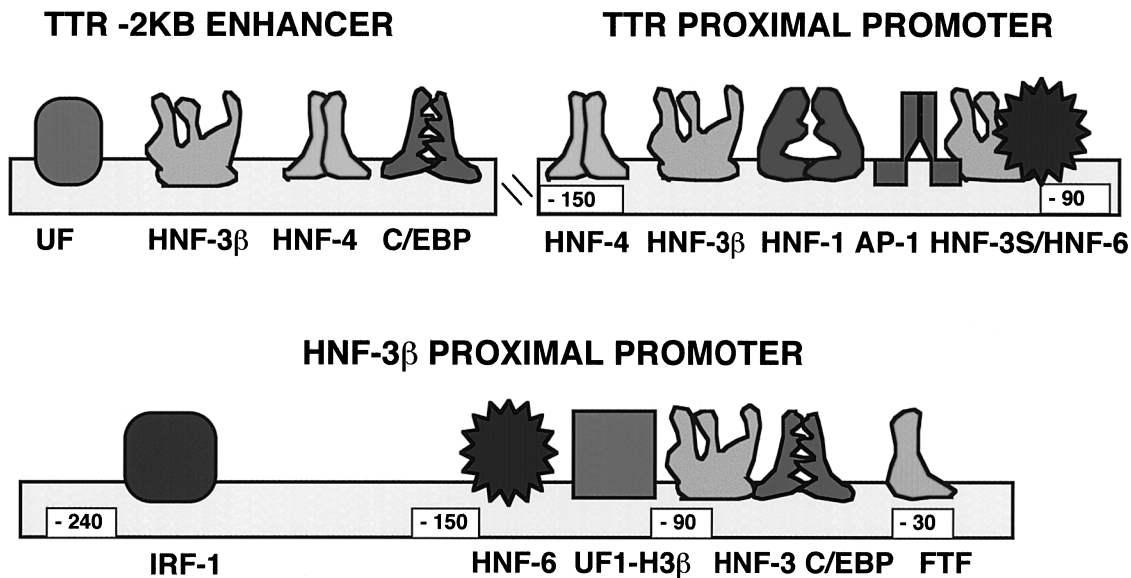


FIGURE 5.1. Transcription factors that regulate expression of the transthyretin (TTR) and hepatocyte nuclear factor (HNF)-3 β genes and their binding sites. Schematically shown are the HNF-3 β and TTR promoter constructs and their corresponding transcription factors. The TTR regulatory regions are bound by members of four different liver-enriched transcription factors HNF-1, HNF-3, HNF-4, HNF-6 and C/EBP (10,24,29,31) and the growth factor inducible AP-1 protein (30). The strong affinity HNF-3 binding site (HNF-3S) overlaps with the HNF-6 binding site in the TTR promoter (10). The TTR enhancer is also recognized by an uncharacterized ubiquitous factor (UF) and contains one HNF-3 binding site which is selectively recognized by the HNF-3 β isoform (31). The HNF-3 β promoter is regulated by three liver and pancreas transcription factors: HNF-3 and HNF-6 as well as by the C/EBP family (9). A third liver and pancreas-restricted orphan receptor family member fetoprotein transcription factor (FTF) (12,13) recognizes a sequence that is identical to the HNF-3 β UF2-H3 β promoter-binding site (130). Two additional binding sites are recognized by generally expressed factors, UF1-H3 β (130) and the interferon (IFN) response factor-1 (IRF-1), which is activated in response to IFN- γ (127,131).

of liver-enriched transcription factors (Fig. 5.1). This assembly of numerous liver transcription factors is a regulatory feature found in most hepatocyte-specific genes and is required to achieve high levels of expression (2). The TTR regulatory region is composed of a proximal promoter region and a distal 100-nucleotide enhancer region located 2 kb from the transcriptional initiation

site, which is sufficient to elicit hepatocyte expression in transgenic mice and in hepatoma cell (HepG2) transfections (24–28). This enhancer region provides a five- to tenfold stimulation in proximal TTR promoter expression (25,27). Transfection analysis of mutations in the TTR proximal promoter region allowed the identification of binding sites for the transcription factors HNF-3

TABLE 5.1. KNOWN AND POTENTIAL HEPATOCYTE NUCLEAR FACTOR-3 (HNF-3) TARGET GENES

Target Gene ^a	Genbank	Position	Sequence
Rat hepatocyte nuclear factor-3β (HNF-3β)	U50407	-97/-86	c c T G T T T G T T T T
Rat hepatocyte nuclear factor-3α	U86584	-466/-477	T T T G T T T A C a a A
Rat hepatocyte nuclear factor-1α	X63959	-21/-32	c A T A T T T A T c C G
Human insulin-like growth factor-I (IGF-I)	S85346	-180/-169	T c T G T T T G C T a A
Mouse annexin V promoter A	g4007573	-21/-32	T T T G T T T A T a T c
Rat IGF binding protein-1 (IGFBP-1)	M84484	-166/-176	T A T G T T T G T c C T
Rat IGFBP-2	M58560	-860/-872	A T T A T T G A C T c c
Human IGFBP-3	M35878	-1,537/-1,526	T T T A T T T A T T T A
Mouse transthyretin (TTR)	M19524	-95/-106	A T T A T T G A C T T A
Human thyroxine-binding globulin	X64171	-131/-120	A A T A T T T A C T a T
Human retinol-binding protein (RBP)	X02775	-547/-558	A A T A T T T G T T C T
Mouse albumin enhancer	U04199	533/522	A A T G T T T G T T C T
Rat α-fetoprotein (AFP)	M18351	-6,091/-6,102	T T T A T T G A C T T A
Mouse transferrin (TFN)	M30819	-73/-84	T g T G T T T G C g C A
Human apolipoprotein B	M19808	+892/+903	A A T A c T G A C T T T
Human apolipoprotein A/CIII	J00098	-535/-546	T g T G T T T A C T C A
Human protein C	U47685	-15/-26	A A T A T T T G C T T G
Human clotting factor IX	X75349	-372/-383	T T T A T T G A T T T c
Rat α-fibrinogen	X02922	-892/-903	A A T A T T G A T g T A
Human clotting factor VIII	U24224	-900/-911	T T T A T T T A T T T T
Human clotting factor VII	U14580	-392/-381	A A T A T T T A C a T c
Mouse complement component C5	M64852	-656/-645	T T T G T T T G T T T T
Mouse plasminogen activator inhibitor-3	U67877	-910/-921	T A T G T T T A T c C T
Human glucokinase	M90297	-797/-784	A c T A T T G A C T g A
Rat phosphoenolpyruvate carboxykinase	K02299	-247/-258	T T g A T T G A C T a A
Rat glucose-6-phosphatase	U57552	-356/-366	T g T A T T T G T T T G
Rat 6-phosphofructo-2-kinase	M26261	-26/-37	A A T A T T T A T T C c
Rat tyrosine aminotransferase	M34257	-3,788/-3,777	c T T G T T T G C T C T
Rat glycogen phosphorylase	M85280	+304/+315	T g T A T T T G T T C T
Rat carbamoylphosphate synthetase I	X90476	-125/-114	A g T G T T T G C T C T
Rat α-amylase (Amy-1)	M14152	-72/-61	T T T A T T G A T T c A
Rat catalase	M25669	-250/-239	c T T A T T T A T T T G
Rat arginase	M17924	-42/-53	T c T G T T T A T c C A
Human tyrosinase	g37506	-484/-495	T T T G T T T A T T T T
Rat cytochrome P-450 CYP2C12	M33544	-49/-38	A A T A T T G A T T T T
Rat cytochrome P-450 CYP2C13	X79810	-53/-42	A A T A T T G A T T c T
Rat cytochrome P-450e	Y00410	-229/-218	T c T G T T T A C T T A
Human cytochrome P-450 CYP2C18	L16869	-692/-703	A A T G T T T A T c C T
Human cholesterol 7α-hydroxylase	M89647	-290/-279	T T T A T T T G T T C T
Rat sodium-taurocholate cotransporter protein (Ntcp)	L76612	-758/-769	T T T A T T G A C T g T
Mouse P-glycoprotein mdr2	M74151	-752/-741	T T T A T T T A T T T T
Mouse organic cation transporter-2	AJ006037	-144/-125	T g T G T T T A T T g T
HNF-3 consensus ^b			W W T R T T T R Y T Y D W W T A T T G A Y T T W

^aShown are the name of the putative HNF-3 target gene, GenBank accession number, position in the gene, and the HNF-3 binding consensus sequence (10,150).

^bNucleotide abbreviations in the HNF-3 DNA binding consensus sequence are as follows: W = A or T, R = G or A, Y = C or T, and D is not C.

Lower case letters represent nucleotides that deviate from the HNF-3 binding consensus sequence.

(-106 to -94 bp) and HNF-4 (-151 to -140 bp) (25). Additional binding sites for hepatocyte transcription C/EBP, HNF-1, HNF-3, and HNF-4 were found in these regulatory regions as well as sites recognized by other widely distributed transcription factors (4,11,25,29-31). Interestingly, two of the HNF-3 binding sites in the TTR control regions are recognized by only the HNF-3 β pro-

tein, suggesting that a subset of hepatocyte-specific genes may be regulated by a distinct HNF-3 isoform (5,31). In the presence of the TTR enhancer region, TTR promoter expression was abolished with a site-directed mutation that disrupted the strong affinity HNF-3 binding site (HNF-3S) and an overlapping HNF-6 recognition sequence (-106 to -94) (10,25,26). HepG2 transfection

TABLE 5.2. KNOWN AND POTENTIAL HEPATOCYTE NUCLEAR FACTOR (HNF-6) TARGET GENES

Target Gene ^a	Genbank	Position	Sequence
Rat hepatocyte nuclear factor-3 β (HNF-3 β)	U50407	-139/-127	G A T A T T G A T T T T T
Mouse hepatocyte nuclear factor-4 α	S77762	-363/-375	A T c A T T G A C T T c T
Human growth hormone receptor	AJ131868	-913/-925	T T c A T T G A T T T A T
Human insulin-like growth factor-I (IGF-I)	S85346	-289/-301	G T T A T T G A g T A A G
Rat IGF binding protein-1 (IGFBP-1)	M84484	-278/-266	G C c t T T G A T T T c T
Rat IGFBP-2	M58560	-860/-872	A T T A T T G A C T c c T
Human IGFBP-3	M35878	-1,507/-1,429	G A a A T T G A T c T T T
Human IGFBP-4	Y12508	-2,063/-2,051	G T T A c T G A T T A T T
Human IGFBP-5	U20271	-457/-469	G C a A T T G A C T g T A
Mouse transthyretin (TTR)	M19524	-95/-107	A T T A T T G A C T T A G
Human thyroxine-binding globulin	X64171	-101/-113	c A c A T T G A T T A A T
Human retinol-binding protein (RBP)	X02775	-552/-540	A A T A T T G A C c A g A
Mouse albumin enhancer	U04199	624/636	T g T A T T G A T c A g T
Rat α -fetoprotein (AFP)	M18351	-6,091/-6,102	T T T A T T G A C T T A G
Rat ceruloplasmin	M80529	-483/-471	T T T A T T G A C T A C T
Human protein C	U47685	-692/-704	T C c A T T G A T T g c A
Human clotting factor IX	X75349	-372/-384	T T T A T T G A T T T c A
Rat α -fibrinogen	X02922	-892/-9,034	A A T A T T G A T g T A T
Human β -fibrinogen	X05018	-470/-458	A C T A T T G A T T T T A
Human Apolipoprotein AI/CIII	J00098	-811/-799	A A c A T T G A T c A A G
Human Apolipoprotein B	M19808	+892/+904	A A T A C T G A C T T T A
Mouse α_1 -antitrypsin		-195/-184	T C c A T T G A T T T A G
Rat α_2 -macroglobulin	M23567	-442/-454	G g T A T T G A C T T T A
Human glucokinase	M90297	-797/-78	A c T A T T G A C T g A G
Rat phosphoenolpyruvate carboxykinase	K02299	-247/-259	T T g A T T G A C T A A A
Rat glucose-6-phosphatase	U57552	-547/-359	A A T A T T G A T T T T T
Rat 6-phosphofructo-2-kinase	M26261	-200/-212	G A A A T T G A T T T c A
Rat tyrosine aminotransferase	M34257	-776/-764	T T g A T T G A T T A T T
Rat tryptophan oxygenase	X05145	-220/-208	T C T A T T G A T T T A T
Human ornithine transcarbamylase	D00221	-314/-302	T C A A T T G A T T T T G
Rat α -amylase (Amy-1)	M14152	-72/-60	T T T A T T G A T T c A A
Rat catalase	M25669	-836/-824	c C T A T T G A T T A A A
Rat arginase	M17924	-231/-243	A C A A T T G A C A A T T
Rat serine dehydratase (SDH2)	J03864	-655/-643	T A c A T T G A T T T g G
Rat cytochrome P-450 CYP2C12	M33544	-49/-37	A A T A T T G A T T T T T
Rat cytochrome P-450 CYP2C13	X79810	53/-41	A A T A T T G A T T c T G
Human cytochrome P-450 CYP2C9	L16877	-95/-107	T A a A T T G A C c A A T
Human cytochrome P-450 CYP2C18	L16869	-927/-915	c A T A T T G A T T T A T
Human alcohol dehydrogenase-2	M24308	-150/-162	A A T A T T G A C T T C C
Human cholesterol 7 α -hydroxylase	M89647	-1,370/-1,392	T A T A T T G A a T A T G
Rat sodium-taurocholate cotransporter protein (Ntcp)	L76612	-758/-770	T T T A T T G A C T g T T
Mouse P-glycoprotein mdr2	M74151	-877/-889	c C T A T T G A C T g T G
HNF-6 DNA binding consensus sequence ^b			D H W A T T G A Y T W W D

^aShown are the name of the putative HNF-6 target gene, GenBank accession number, position in the gene, and the HNF-6 DNA binding consensus sequence (10).

^bNucleotide abbreviations in the HNF-6 DNA binding consensus sequence are as follows: D is not C, H is not G, W = A or T, and Y = C or T. Lower case letters represent nucleotides that deviate from the HNF-6 DNA binding consensus sequence.

of TTR promoter constructs that altered the HNF-3S (HNF-3S/HNF-6; -106 to -94) sequence so that it bound either HNF-3 or HNF-6 resulted in a 30% reduction in TTR promoter activity, suggesting that normal TTR gene transcription requires binding of both transcription factors (10). It is interesting to note that HNF-3 and HNF-6 potentially regulate expression of similar set of hepatocyte-specific target genes (Tables 5.1 and 5.2). Mutations in the other proximal HNF-binding sites elicited a 40% to 60% reduction in promoter activity, whereas in the absence of the TTR enhancer region these promoter mutations eliminated TTR transcriptional activity (26). These studies suggest that a minimal number of hepatocyte-enriched transcription factors are required to occupy promoter sites to achieve transcriptional activity.

PHYSIOLOGIC ROLE OF HNF-3 IN MOUSE LIVER AND PANCREATIC FUNCTION

Transcriptional Activity of the HNF-3 Proteins

The HNF-3 proteins are members of a growing family of transcription factors that play important roles in the differentiation of distinct cellular lineages in *Caenorhabditis elegans*, *Drosophila*, rodents, and humans (32). These transcription factors share homology in the winged helix DNA-binding domain that consists of a modified helix turn helix motif, which allows binding to DNA as a monomer (33–35). Amino acid sequences located at both the amino and carboxyl terminus of the HNF-3 winged helix DNA-binding domain are sufficient to mediate nuclear localization (36), suggesting that the winged helix motif also evolved with a nuclear translocation function (36–38). The HNF-3 β protein is a potent activator of gene expression through transcriptional activation domains located at the amino and carboxyl terminus (36,39). HNF-3 proteins may also play a role in hepatocyte differentiation because induction of albumin transcription during hepatic specification coincides with *in vivo* footprinting of HNF-3-binding sites in the albumin enhancer region (40). Moreover, HNF-3 proteins are involved in organizing the nucleosome architecture of the -10-kilobase (kb) albumin enhancer sequences (41–43), and HNF-3 protein exhibited more stable binding to the nucleosome assembled DNA albumin enhancer templates (44). Consistent with the ability of HNF-3 to position nucleosome core particles, *in vitro* transcription studies using nucleosome associated α -fetoprotein (AFP) promoter templates demonstrated that HNF-3 binding diminishes chromatin-mediated transcriptional repression of the AFP expression (45).

Potential HNF-3 Target Genes in the Liver

The rodent HNF-3 proteins regulate expression of numerous genes critical for liver function [reviewed in refs. 2 and 3] (Table 5.1)]. These hepatocyte genes include the serum carrier proteins albumin, AFP, apolipoprotein AI, and transferrin (46–50); the cholesterol 7 α -hydroxylase (Cyp7A) enzyme involved in bile acid synthesis (51–53), and the glucose metabolism enzymes L-type 6-phosphofructo-2-kinase (PFK-2), aldolase B, and glucose-6-phosphatase (54–56). HNF-3 is an important regulator of angiotensinogen, the precursor of the vasoregulator angiotensin II (57), the anticoagulation protein C (58,59) and coagulation factors (Table 5.1) and the cytochrome P-450 enzymes [(CYP2C6) (60); for others see Table 5.1)]. HNF-3 proteins also recognize the insulin response elements of phosphoenolpyruvate carboxykinase (PEPCK), aspartate aminotransferase, and insulin-like growth factor binding protein-1 (IGFBP-1) promoter regions (61–63) and participate in growth hormone activation of the insulin-like growth factor-I (IGF-I) gene expression (64). In support of HNF-3's role in regulating transcription of hepatocyte-specific genes, a hepatoma cell line that expresses a dominant negative HNF-3 mutant specifically extinguished transcription of numerous HNF-3 target genes, specifically albumin, TTR, transferrin, PEPCK, and aldolase B (65).

Cellular Expression Pattern of HNF-3 Isoforms in Mouse Development and Adult Organs

In the mouse embryo, HNF-3 β expression initiates during gastrulation [day 6.5 postcoitus (pc)] in the node, notochord mesoderm, floor-plate neuroepithelium, and in visceral, definitive endoderm and gut endoderm (66–69). HNF-3 α expression initiates 1 day later during mouse gastrulation in the definitive endoderm, the anterior notochord, and the entire gut endoderm and the midbrain floor plate (67–69). During organogenesis, HNF-3 α and HNF-3 β genes are expressed in epithelial cells of the developing liver, esophagus, trachea, salivary gland, lung, pancreas, intestine, and stomach (66–69) (Table 5.3). Furthermore, HNF-3 α is expressed in epithelial cells of the renal pelvis, prostate gland, bladder, and urinary tract (70), and its transcription in the prostate gland is testosterone-dependent (71). Furthermore, retinoic acid-mediated differentiation of F9 embryonic stem cells toward visceral endoderm induces HNF-3 α transcription (72), whereas expression of HNF-3 β is delayed (73). In contrast to the other HNF-3 isoforms, HNF-3 γ expression is absent from the lung, trachea, or esophagus (67), but its expression is restricted to the pancreas, stomach, gut, testis, and ovaries (74).

TABLE 5.3. EXPRESSION PATTERNS OF HEPATOCYTE TRANSCRIPTION FACTORS

Gene	DNA Binding	Mouse Embryonic Expression Pattern	Adult Expression
<i>Hnf3β</i>	Winged helix domain (monomer)	Gastrulation (6.5–8.5 days pc): Definitive, visceral, gut endoderm, notochord, floor plate of neurotube Organogenesis (9–18 days pc): Endoderm-derived epithelial cells of liver, pancreas, lung, intestine, stomach, esophagus, tongue trachea, floor plate, and midbrain	Hepatocytes, epithelial cells of pancreas (acinar and β cells), lung, stomach and large intestine, intestinal crypt
<i>Hnf3α</i>	Winged helix domain (monomer)	Gastrulation (7.5–8.5 days pc): Similar to <i>Hnf3β</i> except <i>Hnf3α</i> expression initiates 1 day later in development Organogenesis (9–18 days pc): Similar to <i>Hnf3β</i>	Similar to <i>Hnf3β</i> except <i>Hnf3α</i> is also expressed in epithelial cells of intestinal villus, renal pelvis, prostate and urinary tract
<i>Hnf3γ</i>	Winged helix domain (monomer)	(8.5 days pc): Foregut and hindgut endoderm Organogenesis (9–18 days pc): Gut endoderm-derived epithelial cells of liver, pancreas, intestine, and stomach; visceral endoderm of yolk sac, testis, and ovaries	Hepatocytes, pancreas, stomach, small intestine, large intestine, testes, and ovaries
<i>Hnf6</i>	One cut-homeodomain (monomer)	Organogenesis (9–18 days pc): Gut endoderm-derived epithelial cells of liver, bile duct, gallbladder, pancreas, and intestine; neural crest cells of dorsal root ganglia and marginal layer and ganglion of retina	Hepatocytes, and epithelial cells of intrahepatic bile duct, common bile duct, gallbladder, pancreatic ducts, and exocrine acinar cells
<i>Ftf</i>	Zinc finger orphan steroid hormone receptor (monomer)	Gastrulation (8 days pc): Visceral endoderm of yolk sac, branchial arch, and neurocrest cells Organogenesis (9–18 days pc): Endoderm-derived epithelial cells of liver, pancreas, and intestine; neural crest cells of marginal layer and in rib primordium	Hepatocytes and pancreatic acinar and ductal epithelial cells; restricted to the epithelial cells of the intestinal crypts
<i>Hnf4α</i>	Zinc finger orphan steroid hormone receptor (dimer)	Blastocyst (4.5 days pc): Primary endoderm Gastrulation (5.5–8.5 days pc): Extraembryonic visceral endoderm cells of the yolk sac Organogenesis (9–18 days pc): Endoderm-derived epithelial cells of liver, pancreas, intestine, and stomach; in mesonephric and metanephric tubules of kidney	Hepatocytes, and epithelial cells of pancreas, kidney, intestine, and skin
<i>Hnf1α</i> LFB1	POU-homeodomain (dimerization uses DCoH)	Organogenesis (9–18 days pc): Gut endoderm-derived epithelial cells of liver, pancreas, intestine, and stomach; extraembryonic yolk sac endoderm; polarized epithelium of kidney following appearance of three nephrons	Hepatocytes, and epithelial cells of pancreas, intestine, stomach, and proximal and distal tubules of kidney
<i>C/EBPα</i>	Basic domain leucine zipper domain (bZIP) (dimer)	Organogenesis (13–18 days pc): Hepatocytes of liver, hippocampus (CA1–CA4 and dentate gyrus), Purkinje cells of cerebellum, neurons of midbrain and forebrain	Hepatocytes and adipocytes; epithelial cells of intestine, pancreas, lung, and skin; adrenal gland, placenta, and myeloid cells
<i>C/EBPβ</i>	bZIP domain (dimer)	Organogenesis (9–18 days pc): Similar to <i>C/EBPα</i> except <i>C/EBPα</i> is expressed earlier than <i>C/EBPβ</i> during hepatic development	Hepatocytes, adipocytes; epithelial cells of intestine, lung, skin, and mammary gland; myeloid cells, testis, ovaries; hippocampus.

Expression references: *Hnf3 α,β,γ* (5,9,66–71,74,83,141,151); *Hnf6* (7,9); fetoprotein transcription factor (FTF) (13); HNF-4 (11,104,152); HNF-1 (14,111); *C/EBP α,β* (115,117,121,153–164). pc, postcoitus.

The HNF-3 β Gene Is Critical for Early Embryonic Development

HNF-3 β is known to regulate notochord transcription of the sonic hedgehog (SHH) gene, which is required for inductive signaling during the formation of the neurotube (75,76). Moreover, ectopic expression of HNF-3 β in the hindbrain/midbrain region of day 8.5 transgenic mouse embryos changes the cellular fate of the dorsal neurotube and converts it to floor-plate neuroepithelium, resulting in severe defects in skull, midbrain, colliculi, and cerebellum formation (77). Homozygous null *Hnf3 β* embryos die *in utero* because of defective formation of the node, notochord, and visceral endoderm, which are required for development of the primitive streak during gastrulation (78,79). Tetraploid rescue of the visceral endoderm defect in *Hnf3 β* $-/-$ embryos restored normal primitive streak morphogenesis, but the embryos failed to undergo proper gastrulation because they were still missing the node and notochord and did not develop foregut and midgut endoderm (80). This *Hnf3 β* $-/-$ embryo defect has thereby precluded examination of *in vivo* function of HNF-3 β in the regulation of its hepatocyte target genes.

Elevated Levels of HNF-3 β in Mouse Hepatocytes Influence Expression of Genes Involved in Bile Acid and Glucose Homeostasis

In recent studies, we have increased hepatocyte HNF-3 β levels in transgenic mice using the -3 kb TTR promoter region to assess the role of HNF-3 β in hepatocyte-specific gene regulation (81). We found that increased hepatocyte expression of the rat HNF-3 β transgene protein disrupted the normal hepatic levels of the endogenous mouse HNF-3 α , -3 β , -3 γ , and HNF-6 transcription factors. Moreover, we found that diminished hepatic expression of the endogenous mouse HNF-3 and HNF-6 genes were specific because the transgenic livers exhibited normal expression of the HNF-1 α , HNF-4 α , C/EBP α , and C/EBP β transcription factors. Postnatal transgenic mice exhibit growth retardation, depletion of hepatocyte glycogen storage and elevated serum levels of bile acids. The retarded growth phenotype is likely due to a 20-fold increase in hepatic expression of IGFBP-1, which limits the biologic availability of IGFs required for postnatal growth. The defects in glycogen storage and serum bile acids coincide with diminished postnatal expression of hepatocyte genes involved in gluconeogenesis (PEPCK) and sinusoidal bile acid uptake [sodium-taurocholate cotransporter protein (Ntcp)] respectively. These transgenic studies represent the first *in vivo* demonstration that the HNF-3 β transcriptional network regulates expression of hepatocyte-specific genes required for bile acid and glucose homeostasis as well as postnatal growth.

Targeted Disruption of the Mouse *Hnf3* Genes Demonstrates that They Regulate Expression of Genes Required for Glucose Homeostasis

Use of *Hnf3 β* $-/-$ deficient embryonic stem (ES) cells to form embryoid bodies (EBs) for *in vitro* differentiation toward visceral (yolk sac) endoderm demonstrates that HNF-3 β is involved in cross-regulating the transcription of the *Hnf3 α* , *Hnf1 α* , and *Hnf4 α* genes and is required for expression of apolipoproteins, aldolase B, pyruvate kinase, TTR, and albumin (82). Furthermore, using the EB differentiation system, Duncan and co-workers (82) demonstrated that HNF-3 β expression is increased in the presence of insulin, which is consistent with HNF-3 β 's role in regulating glucose homeostasis genes. Surprisingly, expression of these HNF-3 target genes is upregulated in *Hnf3 α* -deficient embryoid bodies, suggesting that HNF-3 α negatively regulates the transcription of these genes and opposes the transcriptional stimulatory activity of HNF-3 β protein (82). In contrast, these target genes were normally expressed in *Hnf3 α* $-/-$ livers, suggesting that hepatocytes and visceral endoderm exhibit differences in HNF-3 α regulatory pathways (83,84). The *Hnf3 α* $-/-$ mice fail to thrive, are hypoglycemic, and display reduced pancreatic islet expression and secretion of glucagon (83,84). The hypoglycemic phenotype is likely due to a decrease in pancreatic α -cell expression of glucagon, which is required to mobilize hepatic glycogen (Table 5.4). These results suggest that *Hnf3 α* plays an important role in regulating pancreatic transcription of the glucagon gene, which is critical in mobilizing hepatic glycogen stores for serum glucose homeostasis. The *Hnf3 γ* $-/-$ mice displayed no morphologic defects in the intestine, liver, pancreas, and testis, which normally express abundant levels of *Hnf3 γ* (85). Hepatocytes deficient in the *Hnf3 γ* gene display a 50% reduction in expression of several HNF-3 target genes including PEPCK, transferrin, tyrosine aminotransferase (TAT), and a compensatory increase in the level of *Hnf3 α* and *Hnf3 β* expression (Table 5.4). These results suggest that the *Hnf3 γ* isoform is required to regulate a specific subset of hepatocyte-specific genes, several of which are involved in glucose homeostasis.

PHYSIOLOGIC ROLE OF HNF-6 IN LIVER AND PANCREATIC FUNCTION

Potential HNF-6 Target Genes in the Liver

Functional analysis of the TTR and HNF-3 β promoter regions enabled us to identify a cut-homeodomain transcription factor, HNF-6, which is also involved in regulating the expression of numerous hepatocyte-specific genes required for liver function (10) (Table 5.2). The HNF-6 cDNA was isolated by biochemical purification using a

TABLE 5.4. PHENOTYPE OF HEPATOCYTE TRANSCRIPTION FACTOR HOMOZYGOUS NULL OR KNOCKOUT MICE

Gene	General Phenotype of Homozygous Null (-/-) Mouse	Liver Phenotype of Homozygous Null (-/-) Mouse
<i>Hnf3β</i> ^{-/-} mice	<i>Hnf3β</i> ^{-/-} embryos die <i>in utero</i> by day 10 because they fail to undergo gastrulation; lack formation of node, notochord, visceral endoderm, foregut, and midgut, and exhibit defects in neurotube	Unknown because of <i>Hnf3β</i> ^{-/-} early embryonic lethal phenotype
<i>Hnf3β</i> ^{-/-} EB	Use of null <i>Hnf3β</i> ^{-/-} embryonic stem (ES) cells to form embryoid bodies (EB) <i>in vitro</i> , which differentiate toward visceral endoderm (VE)	Decreased VE expression of <i>Hnf3α</i> , <i>Hnf4α</i> , <i>Hnf1α</i> , apolipoproteins (Apo A1, A2, A4, B, C2), aldolase-B, pyruvate kinase, transthyretin (TTR), and albumin
<i>Hnf3α</i> ^{-/-} mice	All <i>Hnf3α</i> ^{-/-} mice die 4 weeks postnatally and exhibit growth retardation and hypoglycemia caused by decreased in pancreatic expression of glucagon, which is required to mobilize hepatic glycogen; increased expression of hexokinase and IGFBP-1 in postnatal gut	No liver phenotype was noted, which is possibly due to compensation by the <i>Hnf3β</i> and <i>Hnf3γ</i> isoforms but their expression are not elevated in the <i>Hnf3α</i> ^{-/-} hepatocytes
<i>Hnf3α</i> ^{-/-} EB	Use of null <i>Hnf3α</i> ^{-/-} ES cells to form EB <i>in vitro</i> , which differentiate toward VE	Increased VE expression of <i>Hnf3β</i> target genes; no change in <i>Hnf3β</i> expression
<i>Hnf3γ</i> ^{-/-} mice	Deficient <i>Hnf3γ</i> ^{-/-} embryos and adult mice display no morphologic defects	50% reduction in expression of several hepatocyte genes: tyrosine aminotransferase, PEPCK, transferrin (TFN); compensatory increased expression of <i>Hnf3β</i> and <i>Hnf3α</i> .
<i>Hnf6</i> ^{-/-} mice	70% of <i>Hnf6</i> ^{-/-} mice die 2 weeks postnatally from diabetes mellitus exhibiting low serum insulin levels; no ventral pancreas and delay in formation of pancreatic islets, but glucagon producing cells in these regenerated islets are not appropriately organized	<i>Hnf6</i> ^{-/-} mice lack a gallbladder and show perturbed differentiation of intrahepatic bile ducts, which is associated with a cholestatic syndrome; intrahepatic bile duct regenerates in the liver of 30% of <i>Hnf6</i> ^{-/-} mice that survive the postnatal insulin and cholestasis crisis
<i>Hnf4α</i> ^{-/-} mice	<i>Hnf4α</i> ^{-/-} embryos die <i>in utero</i> by day 6.5 with defects in visceral endoderm (yolk sac), which is essential for ectoderm survival and gastrulation; tetraploid rescue of visceral endoderm defect in <i>Hnf4α</i> ^{-/-} embryos restores gastrulation and allows formation of embryonic liver	In tetraploid rescued day 12 <i>Hnf4α</i> ^{-/-} fetal livers—diminished expression of pregnane-X-receptor (PXR), <i>Hnf1α</i> , albumin, α -fetoprotein (AFP), TFN, Apo A1, A4, B, C3, C2, phenylalanine hydroxylase (PAH), L-type fatty acid binding protein, erythropoietin, and retinol-binding protein (RBP)
<i>Hnf1α</i> ^{-/-} mice	<i>Hnf1α</i> ^{-/-} mice die around weaning after massive urinary loss of glucose and amino acids (Fanconi syndrome) caused by renal proximal tubular dysfunction; defective glucose-mediated insulin secretion from pancreatic β cells leading to elevated serum glucose	Hepatic expression of phenylalanine hydroxylase is totally silent in <i>Hnf1α</i> ^{-/-} liver leading to phenylketonuria; <i>Hnf1α</i> ^{-/-} mice exhibit diminished hepatic expression of albumin, α_1 -antitrypsin, and β -fibrinogen, and compensatory increase in hepatic levels of vHNF1
<i>CIEBPα</i> ^{-/-} mice	<i>CIEBPα</i> ^{-/-} mice die from hypoglycemia within 8 hours after birth, fail to store hepatic glycogen; hepatocytes and adipocytes do not store lipid; defects in lung, neutrophils, and eosinophils	Increased hepatocyte proliferation and diminished postnatal hepatic expression of glycogen synthase, gluconeogenic enzymes PEPCK and glucose-6-phosphatase
<i>CIEBPα</i> ^{-/-} adult mice	LoxP targeted <i>CIEBPα</i> gene locus allowed disruption of <i>CIEBPα</i> gene in the adult liver using tail vein injection of adenovirus expressing the Cre recombinase gene (95% of adenovirus infects the liver)	In addition to hepatic glucose homeostasis genes listed above, <i>CIEBPα</i> ^{-/-} liver exhibits diminished expression of bilirubin UDP-glucuronosyl-transferase (jaundiced phenotype) and factor IX

Homozygous null (-/-) mice: *Hnf3β* (78–80); *Hnf3β* and *Hnf3α* deficient embryoid bodies (EB) (82); *Hnf3α* (83,84); *Hnf3γ* (85); *Hnf4α* (105–107); *Hnf1α* (109–111); *CIEBPα* (117,119,121,165,166); and adult *CIEBPα* (124).
IGFBP, insulin-like growth factor binding protein; PEPCK, phosphoenolpyruvate carboxykinase.

functional element from the PFK-2 promoter (8) and the yeast one hybrid selection method using the HNF-6 site from the HNF-3 β promoter region (9). Northern blot analysis demonstrates that HNF-6 expression is restricted to adult liver and pancreas and that it is 9 kb in length. The cut-homeodomain HNF-6 protein is a member of the one-

cut family of transcription factors, which binds to the DNA recognition sequence as a monomer using both cut and homeodomain protein motifs (6,7,9). HNF-6 potentially regulates numerous hepatocyte genes including serum carrier proteins, clotting factors, cytochrome P-450, and detoxifying proteins and those involved in glucose and

amino acid homeostasis and in bile acid synthesis and transport (Table 5.2). Furthermore, HNF-6 inhibits glucocorticoid-mediated activation of PFK-2 and PEPCK in hepatoma cell transcription, suggesting that it plays a role in regulating glucose homeostasis (86). Moreover, hypophysectomized rats exhibited significant reductions in hepatic HNF-6 messenger RNA (mRNA), and its normal expression was restored following 1 week of growth hormone treatment (87). Consistent with this notion, growth hormone activates the signal transducer and activator of transcription 5 (STAT5) protein, which binds to and mediates induction of HNF-6 promoter expression (88). These studies indicate that HNF-6 transcription is regulated by the growth hormone signaling pathway.

Cellular Expression Pattern of HNF-6 in Mouse Development and Adult Organs

In day 9 pc mouse embryos, the one-cut-homeodomain HNF-6 transcription factor is expressed at the onset and during morphogenesis of the liver, gallbladder, and pancreas (7,9). Later in mouse development, HNF-6 mRNA levels are observed in the neurocrest cells of the dorsal root ganglia, marginal layer, and nuclei of the mesencephalon and pons as well as the ganglion cells of the retina (7,9). In the developing liver, HNF-6 is expressed in hepatocytes and in the epithelial cells of the intrahepatic and extrahepatic bile ducts (Table 5.3). HNF-6 expression continues in the developing epithelial cells of the pancreatic ducts and endocrine and exocrine cells of the mouse embryo. At 18 days pc of mouse pancreatic development, HNF-6 expression diminishes in the pancreatic endocrine cells when the definitive islets of Langerhans first begin to be organized (9). This suggests that terminal differentiation of the pancreatic endocrine cells requires the cessation of HNF-6 expression.

Persistent Expression of HNF-6 in Mouse Islet Endocrine Cells Causes Disrupted Islet Architecture and Diabetes

To examine whether the reduction in islet expression of HNF-6 is critical for proper pancreatic function, we generated transgenic mice in which the islet-specific regulatory element from the *pancreatic/duodenal homeobox (pdx1)* gene drives persistent expression of HNF-6 in pancreatic islets (89). In these mice, the HNF-6 expressing islet cells were hyperplastic and have aberrant islet organization with an increase in the number of α , β , and PP (secretes pancreatic polypeptide) cells. The transgenic pancreatic islets fail to express the *glucose transporter 2 (glut2)* gene, which is essential for insulin secretion from pancreatic β cells. The transgenic mice are therefore diabetic and are unable to secrete insulin in response to a glucose challenge. These deficits reveal that downregulation of HNF-6 expression during pancreatic islet cell ontogeny is critical for normal organiza-

tional development of the pancreatic islet cells and for transcriptional regulation of the *glut2* gene, which is necessary to regulate β -cell secretion of insulin.

Targeted Disruption of the Mouse *Hnf6* Gene Causes Diabetes and Defects in Gallbladder and Intrahepatic Bile Duct Formation

Consistent with this important role of HNF-6 in pancreatic function, the *Hnf6*^{-/-} mice display severe defects in pancreatic islet formation and do not form the ventral pancreas during development (90). The *Hnf6*-deficient mice are diabetic and most of them do not survive postnatal development (Table 5.4). *Hnf6*^{-/-} mice do not develop a gallbladder and develop cholestasis from disrupted intrahepatic bile duct formation. Approximately 30% of the postnatal *Hnf6*^{-/-} mice survive these pancreatic defects and form aberrant pancreatic islets displaying abnormal organization of glucagon-producing α cells. The surviving *Hnf6*^{-/-} mice are also able to regenerate intrahepatic bile ducts and thus partially restore normal biliary function. These genetic studies indicate that HNF-6 expression is critical for the formation of pancreatic endocrine cells, intrahepatic bile ducts, and gallbladder.

FUNCTIONAL ROLE OF HEPATIC HNF-1 AND HNF-4 USING HOMOZYGOUS NULL MICE

Human Autosomal-Dominant Form of Non-Insulin-Dependent Diabetes Is Caused by Mutations in the *Hnf1 α* and *Hnf4 α* Genes

In the adult mouse, the POU-Homeodomain HNF-1 α and steroid hormone receptor HNF-4 α transcription factors are expressed in the hepatocytes and epithelial cells of the pancreas, intestine, stomach, and kidney (Table 5.3). The HNF-1 α protein binds to its DNA recognition sequence as a dimer using a myosin-like dimerization domain located at the amino terminus of the protein (14,15). HNF-1 α dimers are stabilized through association with dimerization cofactor of HNF-1 α (DcoH) protein, which is identical to the aromatic amino acid metabolizing enzyme 4 α -carbinolamine dehydratase (91). HNF-1 α will also form heterodimers with a related family member named vHNF-1 (16), whose expression is detected earlier than HNF-1 α in the visceral endoderm of the yolk sac (Table 5.3). The steroid hormone family member HNF-4 protein utilizes a zinc finger domain to recognize DNA either as a homodimer or as heterodimer with retinoic X receptor α (92–94). Transcriptional activity of HNF-4 has been reported to be modulated through binding of the endogenous ligand fatty acyl-coenzyme A (CoA) thioesters (long chain) and through protein phosphorylation (11,95–97). Numerous hepatocyte-specific genes are known to be potentially regulated by the HNF-1 and HNF-4 tran-

scription factors (98,99). The HNF-1 α and HNF-4 α genes are also mutated in pedigrees of human families suffering from a maturity onset diabetes of the young 3 (MODY-3) and MODY-1, respectively (100–103). These patients exhibit an autosomal-dominant form of early-onset non-insulin-dependent diabetes. Mutation of HNF-1 α and HNF-4 α transcription factors cause non-insulin-dependent diabetes, which indicates that they regulate expression of genes critical for glucose homeostasis.

Targeted Disruption of the Mouse *Hnf4 α* Gene Identifies Glucose Homeostatic Target Genes

In mouse development, *Hnf4 α* is expressed in the primary and extraembryonic visceral endoderm prior to gastrulation (104). During organogenesis, it is expressed in epithelial cells at the onset of liver, pancreas, and intestine formation. Consistent with its early embryonic expression pattern, *Hnf4 α –/–* embryos exhibited a severe visceral endoderm defect preventing gastrulation and they fail to develop past day 6.5 pc (105). Use of *Hnf4 α* null ES cells to form EB *in vitro* to differentiate toward the visceral endoderm cell lineage revealed decreased expression of glucose transporter 2, the glycolytic enzymes aldolase B and glyceraldehyde-3-phosphate dehydrogenase, and liver pyruvate kinase, substantiating the role of HNF-4 α in regulating genes involved in glucose homeostasis (101). *Hnf4 α* -deficient visceral endoderm displays reduced expression of *Hnf1 α* , AFP, transferrin (TFN), several of the apolipoproteins (Apo), TTR, and retinol-binding protein (RBP) (106). Tetraploid rescue of the visceral endoderm defect in *Hnf4 α –/–* embryos restores gastrulation and allowed formation of the liver and other organs to proceed. At day 12.5 pc, *Hnf4 α –/–* fetal liver exhibits diminished expression of the steroid hormone family member *pregnane-X-receptor (PXR)* and *Hnf1 α* genes, the latter of which substantiates its role in cross-regulation of hepatic HNF-1 α expression (107). The disruption of this liver transcriptional regulatory pathway resulted in diminished expression of albumin, AFP, TFN, several distinct apolipoproteins, phenylalanine hydroxylase (PAH), L-type fatty acid binding protein, erythropoietin, and RBP (Table 5.4). In summary, HNF-4 is not required for liver specification but it regulates hepatocyte-specific genes essential for liver function.

Targeted Disruption of the Mouse *Hnf1 α* Gene Causes Non-Insulin-Dependent Diabetes, Renal Dysfunction, and Phenylketonuria

In support of the previously mentioned *Hnf1 α* MODY phenotype, *Hnf1 α –/–* mice exhibit defective glycolytic signaling of pancreatic β cells, resulting in diminished insulin secretion in response to a glucose challenge (108–110). *Hnf1 α –/–* mice died at the time of weaning from the severe wasting Fanconi syndrome (111), which is caused by renal proximal tubular dysfunction leading to massive uri-

nary loss of serum glucose and amino acids (Table 5.4). Hepatic expression of PAH is also completely extinguished in the *Hnf1 α –/–* mice and resembles the human disease phenylketonuria (109,111). Interestingly, inactivation of the *Hnf1 α* gene eliminated liver-specific DNase I hypersensitive sites within the PAH promoter region, suggesting that this transcription factor is involved in chromatin remodeling of the PAH regulatory locus (112). *Hnf1 α –/–* mice also exhibited diminished hepatic expression of albumin, α_1 -antitrypsin, and fibrinogen, and compensatory increase in vHNF-1 levels (109,111). In another knockout mouse study, the *LoxP Hnf1 α* targeted locus was mated to an E11a promoter driven Cre recombinase transgenic mouse to elicit an early embryonic removal of the selectable neomycin gene and first *Hnf1 α* exon sequences. These *Hnf1 α –/–* mice were viable and exhibited minimal renal dysfunction (109). It was noted that the *Hnf1 α –/–* mice displayed non-insulin-dependent diabetes and diminished hepatic expression of IGF-I and IGF-II, leading to a significant reduction in postnatal growth (109). The HNF-1 α protein is involved in transcriptional activation of genes critical for hepatocyte and pancreatic β -cell function but it was not required for specification of these cellular lineages.

Targeted Disruption of the Mouse *vHnf1* Gene Demonstrates Its Essential Role in Visceral Yolk Sac and Gut Endoderm Formation

Consistent with an earlier expression pattern of vHNF1 in the visceral endoderm, disruption of the *vHnf1* gene resulted in an embryonic lethal phenotype due to disorganization of the visceral yolk sac endoderm, which prevented gastrulation (113). Tetraploid rescue of the visceral endoderm defect in *vHnf1 –/–* embryos restores gastrulation and allows mouse embryos to develop until day 10 of gestation, but gut formation is impaired and the mouse embryo does not turn. Use of *vHnf1 –/–* ES cells to form embryoid bodies demonstrates that *vHnf1* is required for visceral endoderm expression of *Hnf1 α* , *Hnf4 α* , *AFP*, *ApoA1*, *ApoA4*, and *TTR* genes and displays reduced expression of the HNF-3 isoforms, transferrin, and GATA-4 transcription factor (113). Taken together these studies indicate that *vHnf1* is critical for specification of the visceral yolk sac and gut endoderm.

C/EBP α REGULATES HEPATIC GENES CRITICAL FOR METABOLIC HOMEOSTASIS

Cellular Expression Pattern of C/EBP α in Mouse Development and in Adult Organs

The CCAAT/enhancer-binding proteins (C/EBP) utilize a basic leucine zipper (bZIP) bipartite DNA-binding domain consisting of a dimerization interface composed of heptad repeated leucine residues termed the “leucine zipper” and a DNA-binding interface consisting of basic amino acids

(114). In mouse development, hepatic expression of C/EBP α is observed by day 13 and significant mRNA levels are also observed in the hippocampus, Purkinje cells of the cerebellum, and neurons of midbrain and forebrain (115). In adult mice, C/EBP α is expressed in differentiated hepatocytes, adipocytes, keratinocytes, and myeloid cells as well as epithelial cells of the lung, intestine, adrenal gland, pancreas, and placenta (Table 5.3). A similar expression pattern is observed with the C/EBP β isoform, except that C/EBP β 's expression initiates later in hepatic development and its levels are also found in epithelial cells of the mammary gland, testes, ovaries, as well as neurons of the hippocampus (Table 5.3). Furthermore, in regenerating and acute-phase liver a transient decrease in C/EBP α levels is observed with compensatory increase in hepatic expression of the C/EBP β and C/EBP δ isoforms (116–119).

Targeted Gene Disruption of Mouse C/EBP α Gene Displays Diminished Expression of Hepatic Genes Critical for Glucose Homeostasis

Because C/EBP was expressed in tissues involved in lipid and glucose homeostasis, McKnight and co-workers (120) proposed over 10 years ago that the C/EBP α transcription factor regulates expression of genes involved in energy metabolism. In support of this hypothesis, C/EBP α $-/-$ mice die from hypoglycemia within 8 hours postpartum due to a complete absence of hepatic glycogen storage and a failure to store lipid in hepatocytes and adipocytes (121). The hepatic glycogen storage defect is due to diminished postnatal expression of glycogen synthase and gluconeogenic enzymes PEPCCK and glucose-6-phosphatase. Consistent with the antiproliferative activity of C/EBP α protein, C/EBP α -deficient liver shows increased hepatocyte proliferation and disruption of the normal liver and lung architecture (117,119). Further characterization of this aberrant proliferation in C/EBP α -deficient hepatocytes demonstrated that C/EBP α stabilizes the cyclin kinase inhibitor p21 protein to inhibit hepatocyte replication (122). Furthermore, C/EBP α regulates activity of a hepatic protease that is involved in generating a transcriptional repressor LIP (liver-enriched transcriptional inhibitory protein), an amino-terminal truncation of the normal C/EBP β protein (123). Moreover, use of adenovirus delivery of cre recombinase to inactivate adult hepatic expression of a loxP targeted C/EBP α gene locus identified other target genes that were not discovered in the straight knockout due to its early postnatal lethality (124). In addition to the glucose homeostatic genes, C/EBP α -deficient adult liver exhibited diminished expression of the bilirubin uridine diphosphate (UDP) glucuronosyltransferase gene causing an increase in serum levels of unconjugated bilirubin leading to severe jaundice (124). Furthermore, the C/EBP α $-/-$ adult hepatocytes exhibited decreased hepatic expression of the blood clotting factor IX gene (124), which was also observed in the homozygous null C/EBP α $-/-$ mice (125). These stud-

ies demonstrate that C/EBP α regulates expression of genes involved in hepatic glucose and bilirubin homeostasis as well as hepatic and adipocyte lipid storage. Other studies identified C/EBP α as an important mediator of neutrophil and eosinophil development (Table 5.4).

EVIDENCE FOR CROSS-REGULATION OF LIVER TRANSCRIPTION FACTOR EXPRESSION

Accumulating evidence suggests that maintenance of hepatocyte-enriched expression of transcription factors involves cross-regulation by one or more unrelated liver-enriched transcription factors (7,9,10,13,70,82,106,107,126–128). Characterization of the HNF-3 β promoter region has allowed the identification of three distinct DNA-binding sites that are essential for HNF-3 β promoter activity and that are bound by the HNF-6, FTF, and C/EBP protein families (7,9,10,13,127). Hepatoma cell transfection studies have demonstrated that HNF-6 and FTF proteins potentiate expression of the HNF-3 β promoter and that their embryonic expression pattern is consistent with the maintenance of HNF-3 β transcription in the developing liver primordium (7,9,13). It is interesting to note that FTF exhibits an embryonic expression pattern identical to that of HNF-6 in the liver and pancreas (13). Based on these experiments, we propose that collaboration among HNF-6, FTF, and C/EBP β is involved in maintaining HNF-3 β promoter expression in embryonic hepatocytes (Fig. 5.2). Additional transcriptional

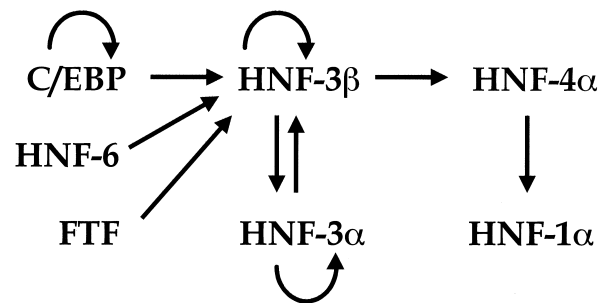


FIGURE 5.2. Cross-regulation of liver transcription factors. Schematically shown is the regulation of HNF-3 β in which *arrows* indicate positive stimulation and *curved arrows* indicate autoregulation. This regulatory scheme is based on transfection data, *in vivo* expression patterns, and on knockout embryoid bodies and mice. We propose that maintenance of the HNF-3 β promoter expression in embryonic and adult hepatocytes is due to collaboration among the cut-homeodomain HNF-6 protein (9,10), bZIP (C/EBP α and C/EBP β) (127), and the orphan receptor family member FTF (12,13). Furthermore, the HNF-3 β protein regulates its own expression (130) and cross-regulates the expression of other liver transcription factors including HNF-3 α (70), HNF-1 α (126), and HNF-4 α (82). Hepatic expression of HNF-1 α is cross-regulated by HNF-4 α (107). Although the HNF-4 α promoter contains HNF-1 α -binding sites (128), its expression was not affected in HNF-1 α -deficient hepatocytes (109,111). Furthermore, normal hepatic expression of HNF-6 expression was observed in HNF-4 α -deficient hepatocytes (107), even though an HNF-4-binding site was present in the HNF-6 promoter region (88).

activation of the HNF-3 β promoter may involve the bZIP family members DBP and C/EBP α (127), whose hepatic expression is observed perinatally and at 13 days of gestation, respectively (115,129). Once HNF-3 is expressed, *in vitro* and *in vivo* studies suggest that an autoregulatory promoter site functions to further activate its own expression (Figs. 5.1 and 5.2) (77,130), which may also be cross-regulated by the other HNF-3 proteins (70). The HNF-3 β promoter also contains functional binding sites for a widely expressed factor, UF1-H3 β (130) and for the interferon regulatory factor-1 (IRF-1) protein (127). *In vitro* and *in vivo* studies demonstrated that the IRF-1 binding site in the HNF-3 β promoter region mediated interferon- γ activation of HNF-3 β expression (127,131).

OTHER TRANSCRIPTION FACTORS REQUIRED FOR LIVER FUNCTION AND DEVELOPMENT (SEE CHAPTER 1)

Tissue-Specific Transcription Factors Involved in Liver Development

In vivo footprinting studies by Zaret (40) have shown that GATA-4 and HNF3 binding sites in chromatin are occupied on the albumin enhancer region prior to hepatic specification, suggesting that the GATA-4 transcription factor may be involved in liver development. Consistent with this notion, in addition to the disruption of heart tube formation, GATA-4 deficient embryos exhibit severe defects in foregut morphogenesis, which gives rise to the presumptive liver (132,133).

Mesenchyme–epithelial interactions using paracrine and cell-cell contact play an important role in mediating organ morphogenesis. The homeodomain *Hlx* gene is expressed in the visceral mesenchyme of the developing liver, gallbladder, and intestine. Consistent with a role of Hlx in regulating genes mediating mesenchyme–epithelial morphogenic signaling, homozygous *Hlx* null mouse embryos die *in utero* and display severe inhibition in proliferative expansion during liver and intestine morphogenesis (134).

Proliferation-Specific Transcription Factors Involved in Liver Development

A number of proliferation-specific transcription factors and DNA repair enzymes play an important role in liver development. Mice deficient in *c-jun* display an embryonic lethal liver phenotype in which day 13 pc fetal livers exhibit extensive apoptosis of both hematopoietic cells and hepatoblasts (135). Liver degeneration and apoptosis were also observed in mouse embryos deficient in the *I κ B* kinase- β gene, which encodes the kinase responsible for activation of NF κ B through phosphorylation of I κ B (136). Mouse embryo hepatocytes deficient in the XBP-1 transcription factor (bZIP family member) displayed significantly reduced growth rate and prominent apoptosis leading to an embryonic lethal phenotype (137). Targeted disruption of

the *heavy metal-responsive transcriptional activator (MTF-1)* gene, which encodes a transcription factor regulating basal and heavy metal-induced expression of metallothioneins leads to an embryonic lethal phenotype displaying impaired development of hepatocytes (138). Mouse hepatocytes that are disrupted in the *excision repair cross-complementing (ERCC)-1* gene are prematurely polyploid, and ultimately these knockout mice die perinatally from liver failure (139). Clevers and colleagues have shown aberrant embryonic polyploid phenotypes in cardiomyocytes and hepatocytes of mouse embryos deficient in the proliferation-specific winged helix transcription factor, HFH-11/Trident (140,141), and the mice die immediately after birth (142). These results indicate that loss of HFH-11/Trident function causes the uncoupling of DNA synthesis from mitosis and suggest that HFH-11/Trident may regulate genes involved in cell cycle checkpoint control. The fact that premature expression of HFH-11/Trident in regenerating liver accelerates the timing of both hepatocyte DNA replication and mitosis further supports its role in cell-cycle regulation (143).

Steroid Hormone Receptors Regulates Transcription of Cholesterol 7 α -Hydroxylase Gene in Response to Cholesterol Metabolites (See Chapter 16)

The liver plays a central role in cholesterol synthesis via transcriptional regulation of key cholesterol biosynthetic genes by the sterol regulatory element binding proteins (SREBPs), which are membrane-bound transcription factors that are activated by proteolysis in response to diminished membrane cholesterol content (144). Hepatocytes also metabolize cholesterol into bile acids, which are released into the digestive tract for digestion of lipids and as the major pathway for eliminating cholesterol from the body (145). Cholesterol 7 α -hydroxylase (Cyp7A) is the rate-limiting enzyme involved in hepatocyte-specific synthesis of bile acids from cholesterol, and its transcription is stimulated by cholesterol metabolites and repressed by bile acids (145). Recent studies demonstrated that the steroid hormone family members, liver X receptors (LXRs), are activated by oxysteroid ligands (oxidized derivatives of cholesterol) and that LXR transcriptionally activates Cyp7A promoter expression (146). Consistent with a critical role in cholesterol metabolism, LXR-deficient mice are unable to induce transcription of the Cyp7A gene in response to dietary cholesterol. The *LXR*^{-/-} mice accumulate excessive amounts of cholesterol in the liver and develop impaired hepatic function (147). Conversely, bile acids function as endogenous ligands for the farnesoid X-receptor (FXR), which binds to the Cyp7A regulatory region and mediates its transcriptional repression in response to increased hepatic bile acid levels (148,149). Bile acid transcriptional control of the Cyp7A gene by FXR thereby mediates regulation of de novo hepatocyte synthesis of bile acid. These

studies demonstrate that the steroid hormone receptors regulate transcription of the *Cyp7A* gene, which encodes the major enzyme involved in synthesis of bile acid from cholesterol, through ligand binding of metabolites of the cholesterol-bile acid metabolic pathway.

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